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THE SUGARCANE FISH SILAGE FERMENTATION*

Prepared by

D.A. Ali**

N. Samaroo***

L.A. Shah****

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** Doctor, Associated Director, Caribbean Industrial Research Institute (CARIRI), Tunapuna Post Office, Trinidad and Tobago.

*** Doctor, Researcher, Caribbean Industrial Research Institute (CARIRI), Tunapuna Post Office, Trinidad and Tobago.

**** Doctor, Researcher, Caribbean Industrial Research Institute (CARIRI), Tunapuna Post Office, Trinidad and Tobago.

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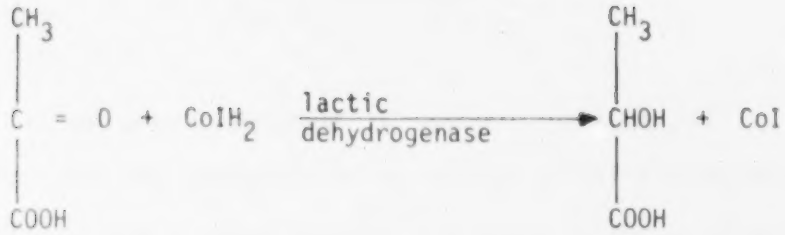
I. BACKGROUND

The ensiling of grass in temperate countries is mediated by a lactic process which preserves the material. Attempts to ensile sugarcane produces ethanol-acetic acid, because it is mediated by yeasts which are selected by the high sucrose content. The incorporation of fish in the fermentation (C:N ratio 15:1) with animal droppings as inoculum redirects the fermentation to produce high levels of lactate which inhibit proteolysis and lead to a satisfactory silage which could be stored for up to twelve weeks.

II. INTRODUCTION

The process of ensiling whereby grass harvested in the summer can be stored and used in the winter for ruminant feeding is a well-established technology in temperate countries. Successful ensiling requires the presence of suitable microflora, adequate substrates and a physical environment conducive to the fermentation process (McCullough 1978). Generally speaking, grass is harvested in the late summer months, packed tightly in tall airtight containers or buildings (silos) and allowed to ferment. If the grass has a low dry matter (DM) content, low soluble sugars, or a low population of lactic acid bacteria, dramatic improvements in the quality of the silage may be obtained by addition of lactic bacteria or low-grade syrups (McDonald *et al*, 1964; Ohyama *et al*, 1975). A rapid fermentation ensues with the degradation of soluble sugars to pyruvate and then further to lactate:



The lactate so formed rapidly reduces the pH of the material, thereby preventing proteolysis and further deterioration of the material. This silage can be stored for several months. It has high acceptability by animals and its nutritional quality is high.

In many parts of the tropics, sugarcane is an important grass that could have implications for ruminant nutrition, both as fresh and ensiled material. Unlike most grasses which are high in protein and low in carbohydrate, sugarcane is low in protein (<1.5%) but high in soluble sugars (>15%). On storage, therefore, sugarcane undergoes a rapid 2C fermentation mediated by yeasts via the Emden-Meyerhoff pathway to produce high amounts of ethanol which become converted to acetic acid by various bacteria (Ravelo *et al*, 1977).

The reasons for wishing to ensile sugarcane have previously been stated by Preston and his co-workers (Preston *et al*, 1976). These relate to problems with wet season harvesting of the crop and the fact that nutritive value is highest in the dry season. Preston *et al*, (1976) have reviewed the work on sugarcane ensiling and reported poor performance of animals fed the material due to reduced voluntary intake and poor feed conversion, the results of the presence of alcohol, acetic acid and the very low pH.

While reviewing such work as discouraging thus far, Preston *et al*, (1976) have stated that in the presence of adequate amounts of nitrogen,

less alcohol would be formed since the environment would be unfavourable to yeast development.

This work attempts to redirect the normal 2C-fermentation of stored sugarcane to a 3C-fermentation. Waste fish is incorporated as a source of nitrogen with fresh ruminant manure providing the inoculum. It is hoped both to preserve the fish protein for subsequent nutrition and also produce an acceptable silage.

III. METHODS AND MATERIALS

Sugarcane was crushed using a CREMASCO Mill. The sugarcane used was variety HJ 5741, approximately one year old obtained from a nearby sugar company. After the whole cane, free of tops and roots, was crushed, it was packed in ice and transported back to the laboratory for immediate use. Frozen ocean perch was transported to the laboratory in the frozen condition. At first, frozen perch in the round were crushed in CREMASCO, but this gave rise to a paste which was difficult to manipulate. Better results were obtained by chopping up the fish into small cubes of side *ca.* 1 cm. Fresh cattle manure was obtained from a nearby animal farm.

Various analyses were carried out as detailed in ASTM (1974); Friebold & Aruand (1969); Pearson (1970) and Youssef (1977). These included:

a) Carbon: A dried sample was placed in a combustion tube and inserted into a furnace at 1100°C. A stream of CO_2-free oxygen was passed over the combustion tube and the evolved CO_2 was collected

on Ascarite. CO_2 formed was obtained by weight difference of Ascarite.

b) Carbon Dioxide: A sample of headspace gas was withdrawn from the sample bags or the silos and then injected into a Gas Chromatograph (Pye Unicam Model 104). The resulting chromatogram was then compared with the chromatogram of a reference gas of known CO_2 content.

c) Crude Fibre: A moisture-free and ether-extracted sample was digested first with a weak acid solution and then with a weak base solution. Digestion with boiling dilute acid hydrolyses carbohydrate and protein while digestion with dilute alkali saponifies residual fatty materials. Both treatments contribute to the solution of mineral matter. The residue consisting mainly of fibre and a little mineral matter was collected in a filter crucible, dried and weighed. It was then ignited to 600°C to constant weight. Loss of weight is equivalent to crude fibre.

d) Dry Matter: The material was dried in an oven at 150°C to constant weight.

e) Fat (Ether Extract): Ether was continuously volatilized, then condensed and allowed to pass through an oven-dried sample. The extract containing ether soluble materials was collected in a pre-weighed flask. On completion

of the process (12-16 hrs), the flask with the ether extract was dried and the ether-extractable fat determined by difference.

f) Lactic Acid: The method used was that of Bohannon *et al*, (1978). The method depends on derivitization to the methyl ester followed by gas chromatography. We used a Pye Unicam Model 104 with dual FID detectors.

g) Nitrogen and Crude Protein: The nitrogen compounds in the sample were converted to $(NH_4)_2SO_4$ by digestion with conc. H_2SO_4. The acid digest was cooled, then diluted to a known volume (250 ml). 5 ml aliquots of the digest were made strongly alkaline with NaOH. NH_3 was released by steam distillation in a Markham still and collected in boric acid which was then titrated with standard HCl. Crude protein was obtained by multiplying N x 6.25.

IV. RESULTS

The first series of experiments were set up in clear QVF glass cylinders in order to observe the gross effects on the ensiled material. The cylinders used were *ca.* 75 cm tall x 15 cm diameter. The following experiments were set up:

a) Crushed cane only

b) Crushed cane containing finely chopped fish (*ca.* 42 kg cane + 13 kg fish). The sample was well mixed before packing into

the experimental cylinder. C:N ratio was *ca.* 15:1.

c) Same as (b) but containing 1.0% (by net weight) of fresh cattle droppings.

In all the experiments, the material was tightly packed, the cylinders were closed off at the top and left at ambient temperature (approx. 23-25°C).

All samples showed (Fig. 1) a rapid decline in pH. In the samples with cane only, the final pH obtained after about nine days was much lower (3.1) than the final pH obtained in the experiments containing fish (3.8) and fish and animal droppings (4.1).

The experiments were kept going for twelve weeks and then opened for examination. In all the cylinders, there was a partial drying of the surface layers and the development of myceliate fungus at the top (*ca.* 7 cm). The cylinder with the crushed cane had a strong smell of ethanol and acetic acid, but the material appeared well preserved. The cylinder with the cane and fish had no smell of proteolysis and, in fact, had a pleasant, sweet odour. Similarly, in cylinder (c), i.e. crushed cane containing fish and animal droppings, there were no signs of proteolysis and the material appeared well preserved with a sweet, esterified smell. In both cylinders containing the fish, the small bits of fish, bone and fins appeared to have been solubilised.

Samples of each silage were extracted by pressing in a Carver Press and the extracts examined by gas-chromatography for the presence of lactate. The restuls shown in Fig. 2, 3 and 4 illustrate the actual tracings and show little lactate in the cane only silage, but increasing amounts of lactate in cane plus fish and the highest level in cane plus fish plus animal droppings.

In order to carry out repetitive sampling on the silages, it was necessary to repeat the experiments in such a way to exclude the admission of air to prevent aerobic deterioration of the material (Moon *et al*, 1980). The experiments were, therefore, repeated with small amounts of each silage (approx. 400 gm) being tightly packed into small black polythene bags, excess air was evacuated and the bags were securely fastened. The bags were incubated at ambient temperatures (23-25°C).

The entire contents of a bag were used as a sample for the various examinations as described in Table 1. The contents of the bag were divided up into various sub-samples and examined as previously described.

The results from Table 1 show:

a) No net degradation of the fish protein; thus maintenance of the C:N ratio.

b) Early rapid release of CO_2 into the headspace gas followed by a slower release.

c) No changes in crude fibre.

TABLE 1

ANALYSES OF VARIOUS SUGARCANE SILAGES

	CANE + FISH + DROPPINGS				CANE + FISH				CANE ONLY			
	96 hr	3 wk	6 wk	9 wk	96 hr	3 wk	6 wk	9 wk	96 hr	3 wk	6 wk	9 wk
Dry Weight, gm %	20.53	18.34	19.94	16.46	20.21	19.39	18.62	18.70	21.04	17.27	18.34	16.83
Fat, %	1.32	2.75	1.58	1.87	2.28	2.04	2.08	1.40	0.53	1.48	1.62	1.14
Crude Fibre, %	25.6	36.8	33.4	45.3	24.4	39.3	36.9	37.9	30.6	51.1	49.6	48.5
Crude Protein % Dry Weight	17.68	25.57	21.01	25.10	21.03	14.18	30.24	22.10	2.09	2.20	2.07	3.33
N_2 % Dry Weight	2.83	4.09	3.36	4.01	3.36	2.27	4.83	3.53	0.33	0.35	0.33	0.53
Carbon, %	--	46.4	46.5	45.2	--	44.9	42.7	46.6	--	45.4	45.6	46.3
C : N	--	11.4 :1	13.9: 1	11.3:1	--	19.8:1	8.8:1	13.2:1	--	129.7:1	138.3:1	87.3:1
CO_2 %	9.37	--	0.13	0.16	7.61	--	0.06	0.19	8.26	--	0.10	0.10

Microscopic examination of the silages at 96 hrs and 6 wks showed:

	Cane	Cane + Fish	Cane + Fish + Droppings
Unicellular Yeasts	++++	++	++
Budding Yeasts	++++	+	+
Gram Negative Rods	+++	++	++
Gram Positive Sporing Rods	++	++	++
Gram Positive Cocci	+	+	+
Gram Positive Elongated Non Sporing Rods	+	+++	+++

V. DISCUSSION

Various techniques have been tried in sugarcane ensiling to inhibit yeast conversion to alcohol and subsequent acetification. These have included the use of additives such as formaldehyde or formic acid to reduce fermentation activity (McDonald *et al*, 1969), the use of ammonia and urea as buffers (Alvarez and Preston, 1976) and ensiling in the presence of various forages rich in urease (Gill and Munoz, 1981). These techniques have generally improved the quality of the silages ranging from no alcohol production but low lactic acid (Preston *et al*, 1976) with ammonia to no alcohol production with higher pH^S (Gill and Munoz, 1981) with various forages.

In all the work reviewed thus far, levels of lactate have been low and this may explain why such silages have not improved animal performance as was expected (Silvestre *et al*, 1976).

The present work incorporating waste fish and fresh cattle manure provides a novel approach to the problems associated with sugarcane ensiling. The manure provides a rich source of microbes above and beyond the wild yeast flora of the cane while the nitrogenous base of the fish provides the necessary biochemical backup to force a pyruvate-lactate fermentation. The technique appears to provide a viable means of ensiling sugarcane while simultaneously preserving the fish protein.

The work is now continuing in various directions including:

- microbiology of the fermentation
- characterization of fatty acids
- amino acid profiles of the material
- ruminant feeding
- use of alternative waste protein materials.

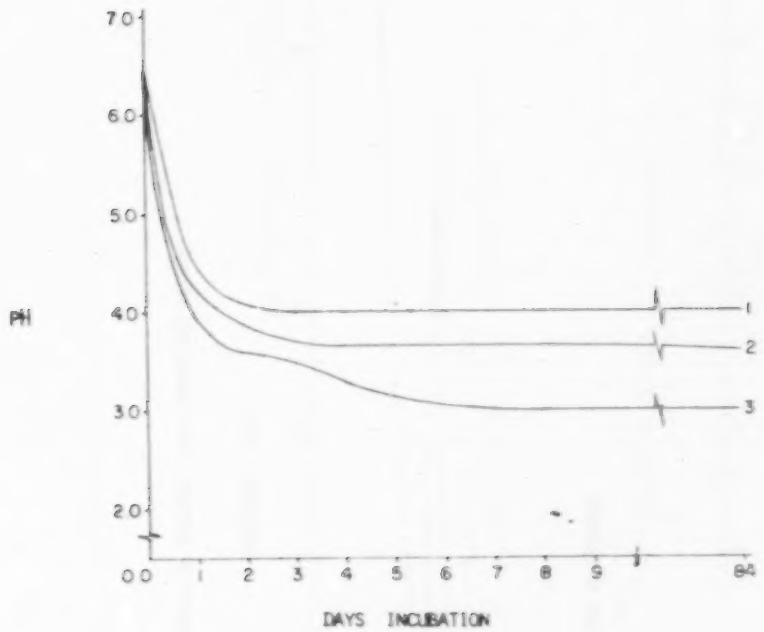


FIG. 1 PH CHANGES DURING ENSILING OF SUGARCANE

1. SUGARCANE + FISH + ANIMAL DROPPINGS
2. SUGARCANE + FISH
3. SUGARCANE ONLY

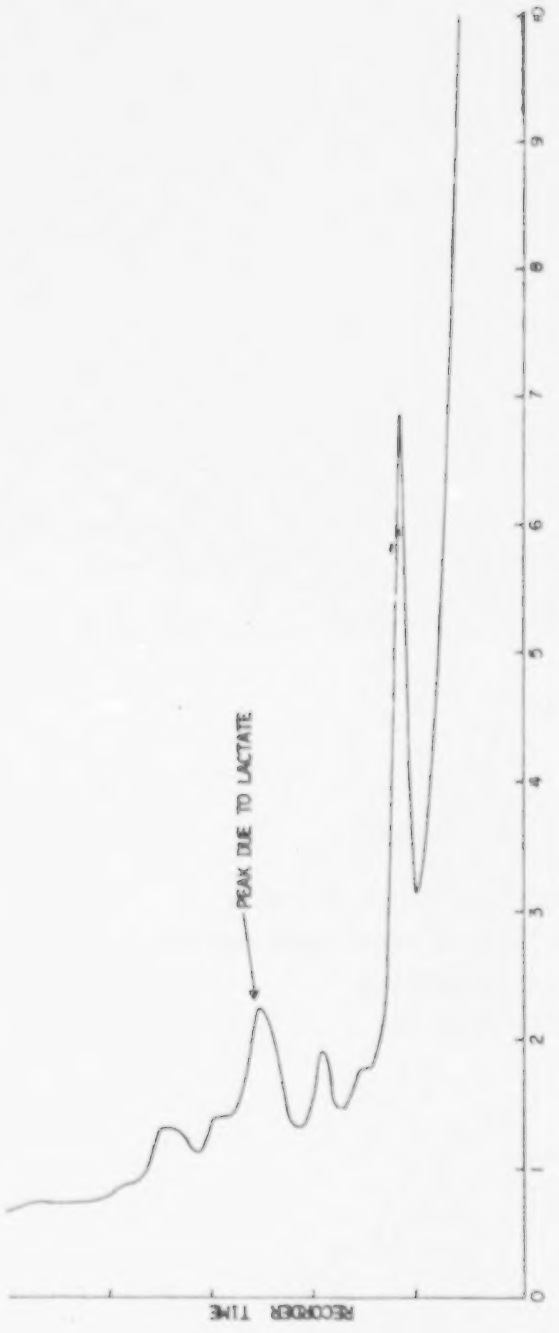


FIG. 2 GAS CHROMATOGRAPH OF EXTRACT FROM SUGARCANE SILAGE

FIG. 3 GAS CHROMATOGRAPH OF EXTRACT FROM SUGARCANE/FISH SILAGE

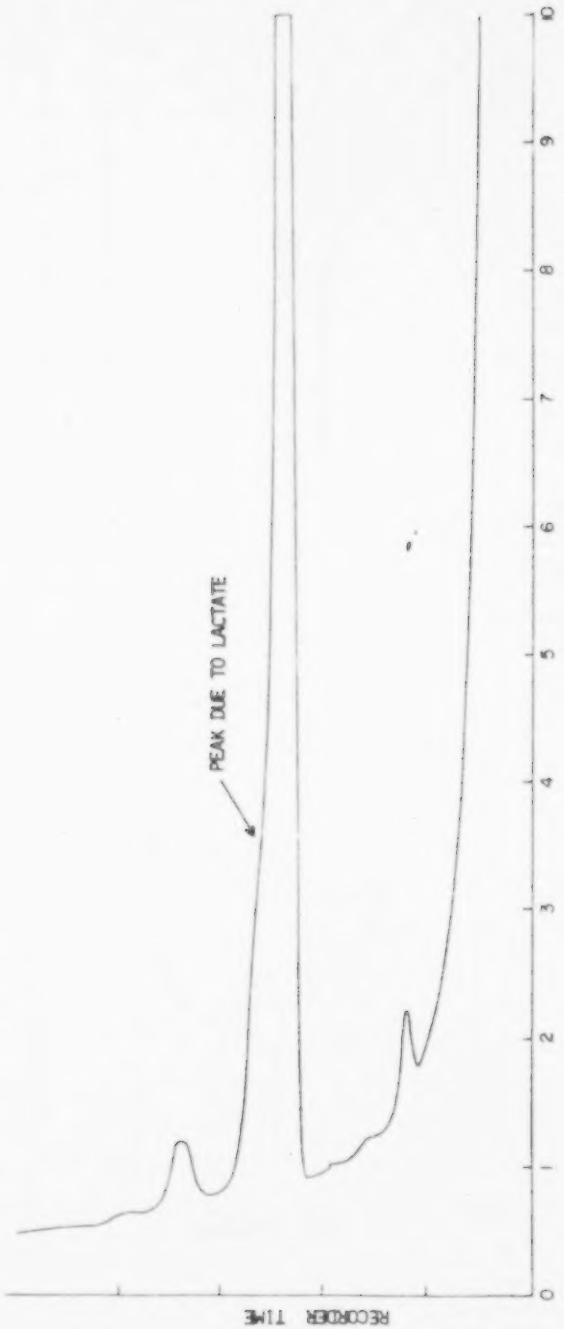


FIG. 4 GAS CHROMATOGRAPH OF EXTRACT FROM SUGARCANE/FISH/ANIMAL DROPPINGS SILAGE

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